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13. ABSTRACT ***(Maximum 200 Words)***

Malaria poses major threat to public health worldwide. The situation is worsened by the rapid emergence of strains resistant to existing drugs. There is a tremendous need to develop new potent drugs for combating this disease. It is therefore important identify novel target pathways or enzymes in the parasite that is essential for the survival and growth of the parasite. Moreover, the targets have to be different in biochemically and structurally so that they present enough selectivity. During the intraerythrocytic stage, glycolysis serves as the major energy producing mechanism for the parasite. LDH, the last enzyme in this pathway, is apparently essential for the survival of *Plasmodium falciparum*, the most lethal strain of human malaria parasite. In this project we explore the possibility that *P falciparum* LDH could be used as a target for developing anti-parasitic agent. Our approach involves high throughput screening of diverse combinatorial library and structure-based design. The high throughput screening effort (40,00 compound diverse representative library) has produced several hits which will be tested further analyzed by *in vitro* enzyme assay and anti-parasitic activity in parasite culture. Selected compounds will be used for structure analysis that will guide further synthesis. Molecular modeling has also produced some interesting results that are presently being tested. We have also expressed, purified, crystallized a new enzyme of the glycolytic pathway which will be studied by X-ray diffraction method.

14. SUBJECT TERMS
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FOREWORD

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N/A In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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N/A In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

[signature] 7/13/00

PI - Signature Date

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INTRODUCTION

Considering the rapid development of resistance in the strains of malaria parasite towards existing drugs, there is an urgent need for discovery of new antimalarial drugs [1]. Glycolytic pathway may provide an useful target for development of chemotherapeutic agent to combat the parasite. Malaria parasite spends much of its life within the red blood cell of the host. During this intraerythrocytic stage, the parasite depends on anaerobic metabolism of glucose for ATP production. As a result, the infected red blood cells exhibit up to a 100 fold increase in glucose consumption as compared to normal erythrocytes and a consequent presence of higher levels of several glycolytic enzymes [2-4]. For glycolysis the parasite utilizes its own set of enzymes [5]. Lactate dehydrogenase (LDH) is the last enzyme in the pathway. It is involved in the regeneration of NAD. Inhibitors of *Plasmodium falciparum* LDH (*Pf*LDH) killed the parasite in culture suggesting that *Pf*LDH is an essential enzyme for the survival of the parasite [5]. *Pf*LDH has several unique features in its sequence and possesses distinct biochemical properties [6, 7]. More importantly the *Pf*LDH structure also presents several characteristic features that can be exploited for the rational design of specific inhibitors [8]. We have proposed to identify a potent and specific inhibitor of *Pf*LDH by a structure-based approach in conjunction with screening diverse chemical library. We also proposed to examine if other enzymes in the glycolytic pathway possess enough specificity for selection of inhibtors.

RESEARCH ACCOMPLISHMENTS

Specifc aim 1: a) Search for selective inhibitors of *Pf*LDH by screeening large and diverse combinatorial library

A combinatorial library of 40,000 compounds containing representatives of every structural class in a larger library was screened in a highthroughput *in vitro* enzyme assay developed in our laboratory. The library was obtained as DMSO solution of representative compounds spotted on 96 well plates. The assay was based on measuring utilization of NADH during conversion of pyruvate to lactate by *Pf*LDH.

Results were analyzed and the compounds were grouped into subsets that exhibited 40-70% activity at 10 μM final concentration. These results are summarized in appendices I-III. As seen in the plots several compounds exhibited 70% inhibition.

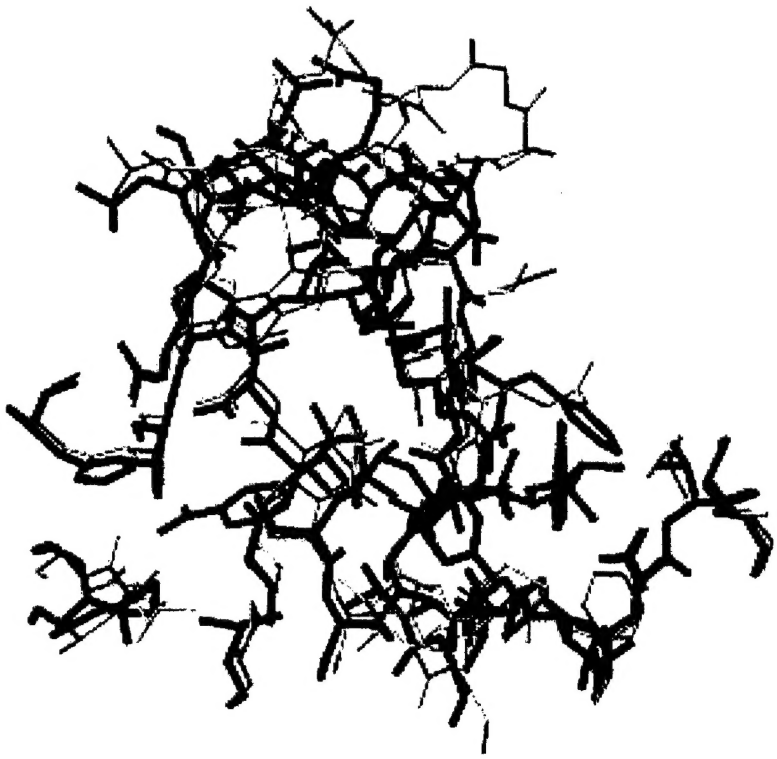
b) Structure-based approach to designing *Pf*LDH inhibitors.

Description of the three dimensional structure

The *Pf*-LDH tetramer has four subunits and each subunit is composed of two domains: the N-terminal NADH binding domain and the C-terminal catalytic domain. The N-terminal domain containing nearly 150 residues is comprised of typical Rossmann fold formed by six stranded parallel β sheet surrounded by α-helices on both sides. This domain contains the substrate specificity loop which consists of the residues 98-109, that are highly conserved in all known LDHs, except *Pf*-LDH. In *Pf*-LDH not only the sequence differs, but there is also a unique five aminoacid insertion from residues 104 to 108. Additional residues that are conserved in other LDHs, but differ in *Pf*-LDH include S163, I250 and

T246. From the investigation of the active site of LDHs with NAD analogues, it has been shown that the replacement of the carboxamide amino group by methyl or acetyl groups does not hinder the formation of the active ternary complex, but only their kinetic properties are modified [8, 9]. The important residues R171, R109, H195 and D168 for the catalytic mechanism are conserved in all LDHs including *Pf*LDH. On the other hand the uniqueness of *Pf*-LDH compared to other LDHs resides in the numerous amino acid substitutions found in the substrate or cofactor binding positions. Of particular interest is the change of amino acid sequences near the active site in the substrate specificity loop. The residues 98-110 form a flexible loop that closes in the ternary complex to help define the catalytic site. The dimension of the cleft formed near the active site by this loop is 10 X 5 X 8 . This loop together with the co-enzyme binding-site was assumed as the target for rational drug design using the program DOCK [10]. The superposition of the *Pf*-LDH (gold) with Pig-LDH structure (blue) is shown in Fig.1 below representing all the residues within 7 distance from the co-enzyme NADH (green). At the nicotinamide end of co-enzyme the substrate specificity loop is unique in *Pf*-LDH structure with the residues Thr101, Lys102, Asp108A and Asn 241 contributing to the sides of the cleft. In the structure based drug design, the central assumption is that good inhibitors must possess significant structural and chemical complementarity to their target receptor. Our basic approach to explore the docking of two objects uses a set of algorithms that develop a molecular surface for the receptor, produce the negative image of the receptor site, and match the molecular structure of a potential ligand to the negative image of the receptor. A large collection of molecular structures such as Cambridge Structural Database and chemicals directory contains a number of diverse molecular shapes. Given a macromolecular receptor for which the structure is known at the atomic level, and a potential ligand from the small molecular database, an automated program developed by Lorber et al [11] was used to dock the potential ligands in to the receptor binding site. The ACD (Available Chemical Dictionary) database typically has 100,000 to 200,000

molecules, and the goal is to find the novel ligand molecules that complement the binding site.



Pf-LDH yellow Pig-LDH blue

Fig. 1 Superposition of *Pf*LDH and Pig-LDH docking sites. NADH is shown in green.

Creation of molecular surface and generation of receptor spheres

Preparation of Ligand and receptor molecules.

The following steps preprocess the ligands for docking. First, the ligand is extracted from the database and transformed into SYBYL mol2 format. Correct atom types including hybridization states as well as correct bond types are defined. Hydrogen atoms are added to all atoms with reasonable geometry, formal charges are assigned to each atom. The ACD database is divided into small groups of compounds each consisting of 10,000 molecules. The crystal

structure of Pig M4 lactate dehydrogenase was determined by Dunn et al [12] at 2.0 resolution (PDB accession code 9LDB), and the crystal structure of *Pf*LDH was determined by us at 1.9 resolution. Both the structures were transformed into SYBYL mol2 format. Except the protein atoms all other co-ordinates for coenzyme, water and substrate analogue were removed. The docking process includes four steps: 1. Creating the negative image of the receptor, 2. Characterization of the site by spheres 3. Overlaying the negative image with small molecules from the database, 4. Ranking and reviewing the best scoring orientations of the most complementary small molecules.

Briefly, in docking, the negative image of the shape of the receptor is represented by a set of spheres. The spheres are constructed from the Connolly [13] rendering of the molecular surface described by Richards [14]. The program RIBBONS [15] was used to create Connolly dot surface and then it was converted to the DOCK format by using the program ms-dock. The resulting molecular surface was used as input to generate the spheres using SPHGEN in the dock suite of programs. The spheres arranged in different cluster sizes represented all the negative image of the receptor. The negative image was limited to the region of the active site and the proximal part of the substrate specificity loop that differs between the parasite and mammalian enzyme. Total number of spheres representing the co-enzyme binding-site and the substrate specificity loop were 138 and 114 respectively. Visualization and the selection of the spheres were done using the program INSIGHT II [16]. Only those spheres well representing the co-enzyme NADH binding pocket and the extra loop region in the receptor molecule *Pf*-LDH were selected for docking. The 138 spheres from the various clusters for co-enzyme site and 114 spheres for the extra loop region represented the entire binding pocket well. SHOWBOX program was run to automatically enclose all the spheres representing the co-enzyme site and the substrate specificity loop region with the additional margin of 4.5 on all sides.

Generation of Contact and Force-field scoring grids

A steric map was calculated with DISTMAP using limits of 2.4 A for polar atoms and 2.8 A for non-polar atoms and an electrostatic map was calculated with CHEMGRID for the binding site with a grid spacing of 0.3 and a dielectric of 4 and non-bonded cut-off of 10 . With all these parameters set, the flexible docking method was performed in the active site region of *Pf*-LDH and Pig M4-LDH structures. The method is able to find ligands known to bind to specified sites that are complementary in shape to the binding site. From each small database, consisting of 10,000 compounds 300 highest scoring compounds were selected and kept for further calculation. After running against all the database, the top-scoring compounds were rerun to choose the final best scored compounds. The final list of the compounds obtained from the different docking sites has been examined by using interactive computer graphics. The ribbon diagrams show pyrene tetrasulfonic acid molecule docked in the active site of *Pf*-LDH and pig LDH structures.

Fig. 2 Docking of pyrene tetrasulfonic acid in pig LDH (left) and *Pf*LDH (right)

Chemicals

Selected compounds from DOCK analysis were purchased from various chemical suppliers and each of them was dissolved in water or in DMSO. LDH activity in the direction of reduction of pyruvate to L-lactate was measured in 100mM Tris pH 7.5, containing 10mM pyruvate and 1mM NADH . The decrease in absorbance was measured at 340 nm. Compounds were tested at different concentrations ranging from 0.01 mM to 1 mM.

Results and Discussion

The optimized DOCK force-field scores, used to select compounds for assay, are provided in the Lists I and II. The list contains the compounds docked in the NADH binding site and the loop region of *Pf*-LDH structure. The top scoring nucleosides and the organic compounds were selected for assay. The

compounds examined in the enzyme assay are listed in Table I. One of the compounds, pyrene tetrasulfonic acid showed inhibitory activity. The compound was inactive at 10 fold higher concentration against a recombinant preparation of human LDH (C-4).

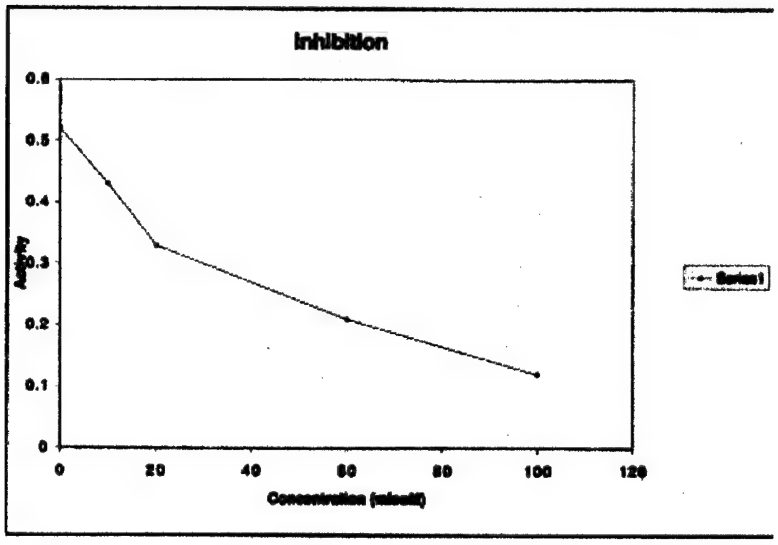


Fig. 3 Plot of *Pf*LDH activity along y-axis at various concentration of pyrene trtrasulfonic acid along x-axis.

Pf:LDH activity was measured for 30 min at 37¡C against various concentration of pyrene tetrasulfonic acid, tetrasodium salt in water. The assay was done using the colorimetric LDH assay kit from Sigma.

The number of compounds obtained from the result of docking of the loop region, involving the cleft in *Pf*-LDH structure, are being tested for inhibition studies. Docking of one compound (2,5-bis-N-4-ethoxyphenyl carbamoyl terephthalic acid hydrate) with *Pf*LDH and pig LDH is shown below in Fig.4.

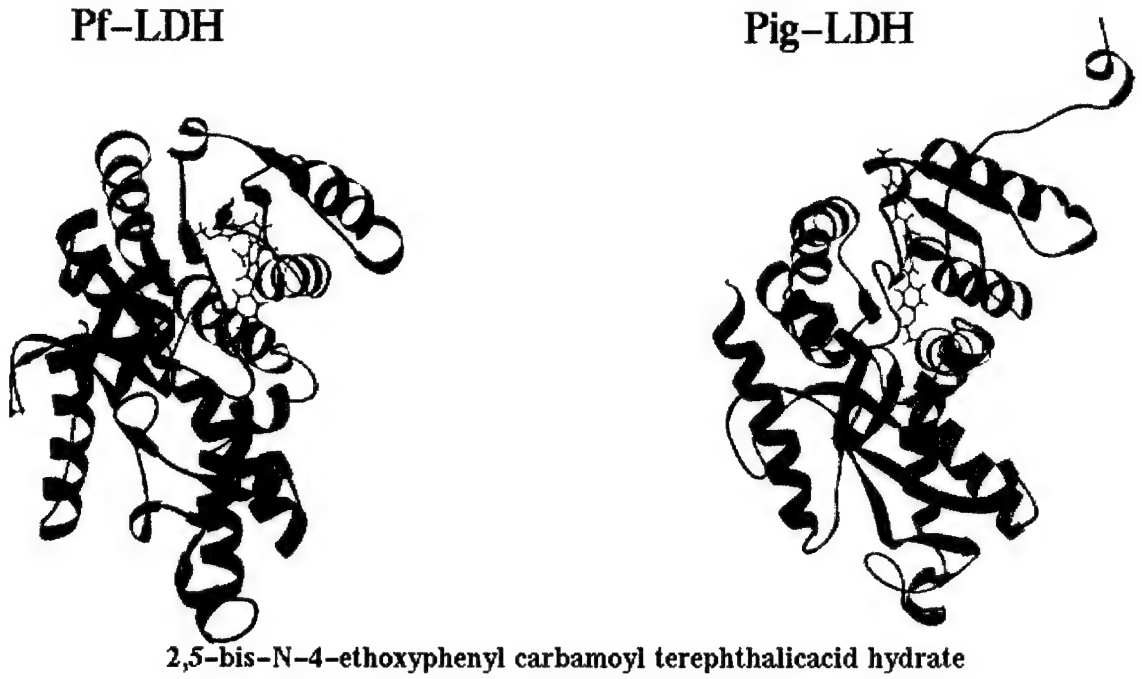


Fig. 4 Ribbon representation of 2,5-bis-N-4-ethoxyphenyl carbamoyl terephthalic acid hydrate docked in *Pf*LDH and pig LDH structures

Specific aim 2: Expression, purification, characterization and crystallization of new targets of *P. falciparum*

Hicks *et al.* (1991) have cloned the 3-phosphoglycerate kinase (*3-pgk*) gene of *P. falciparum.* Previously we subcloned, expressed and purified a recombinant form of 3-pgk with a hexahistidine tag attached at the N-terminus. Recently, we have expressed a new recombinant construct in pET15b vector. Under the control of a T7 promoter, the recombinant 3-pgk was expressed in E. coli BL21(DE3)plysS cells. The protein was purified using immobilized metal affinity chromatography. The hexahistidine tag was removed by treatment with thrombin overnight at 4¡C and subsequent cation exchange chromatography on SP-Sepharose column (Pharmacia). Finally, the protein was subjected to size exclusion chromatography on Superdex75 column. The purified protein migrated as a single band of approximately 46 kD on SDS-PAGE.

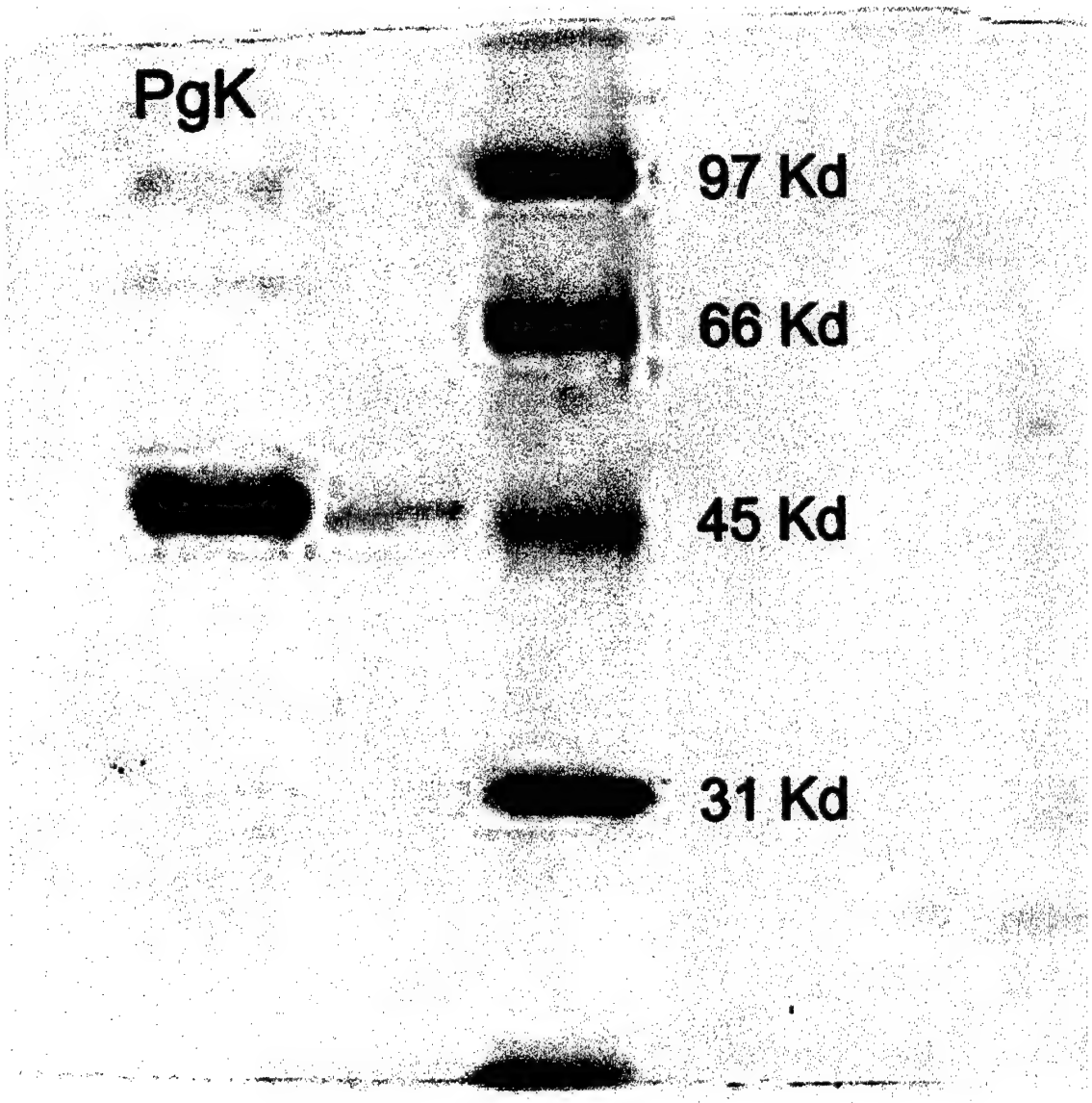


Fig. 5 SDS-PAGE pattern showing purified 3-pgk. Molecular weight standards are shown in the right lane.

The purified 3-pgk was found to be enzymatically active. The protein was active over a wide range of pH with the optimum lying at about pH 7.5. The protein required a divalent cation, activity increased up to 20 mM $MgCl_2$. The Km for ATP and 3-phosphoglyceraldehyde were found to be 0.5 mM and 0.45 mM respectively.

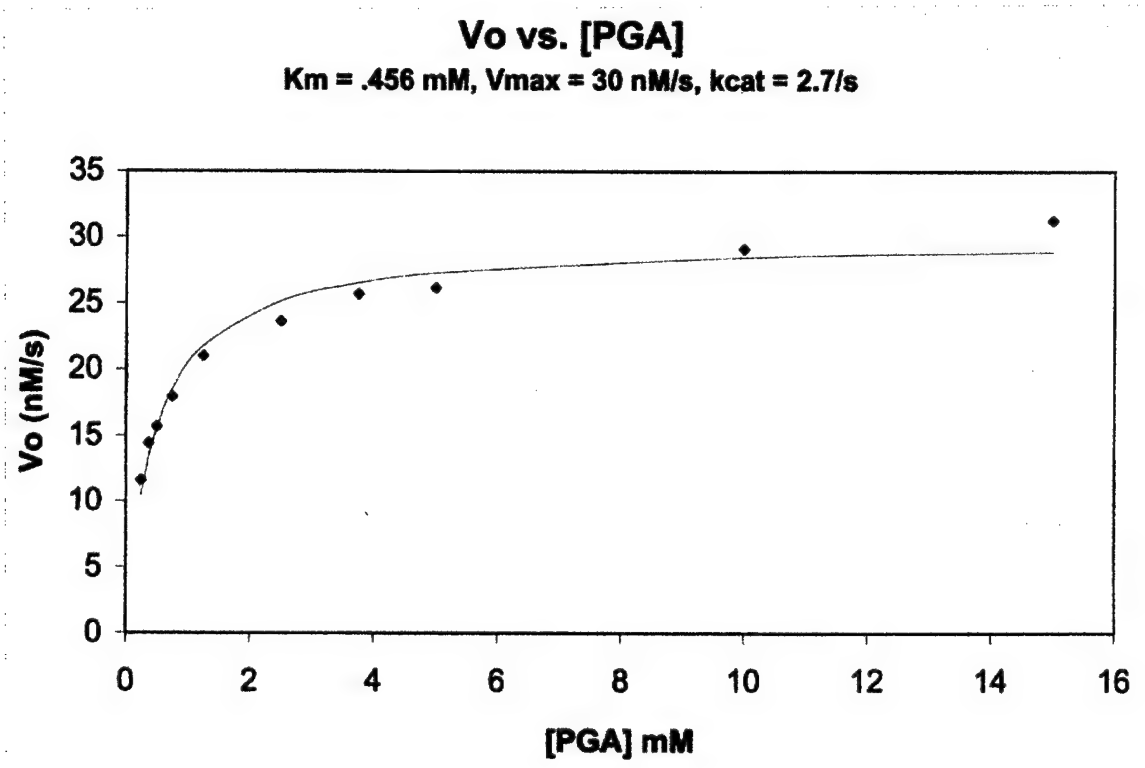


Fig. 6 plot of Vo (y-axis) against 3-PGA concentration. 3-pgk activity was measured as described by Grall et al. (1992) [18].

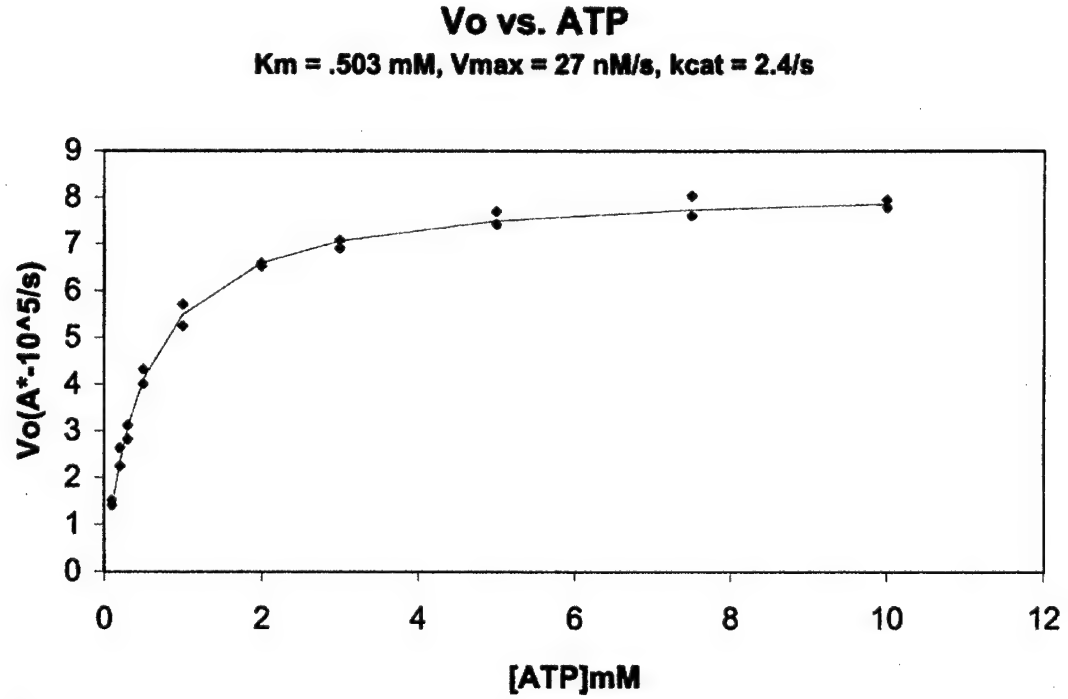


Fig. 7 Km determination. Initial velocity is plotted against various ATP concentration

Crystallization screening of purified 3-pgk yielded small crystals of the protein at room temperature in the presence of 2.5 mM ATP.

We are presently refining the crystallization conditions for growing crystals suitable for X-ray diffraction analysis.

KEY RESEARCH ACCOMPLISHMENTS

We have completed screening a combinatorial library for possible LDH inhibitor. Several potential inhibitors have been identified and selected for next phase of screening.

We have undertaken molecular modeling approach to find selective inhibitor from commercially available chemical libraries.

We have expressed, purified and characterized 3-pgk enzyme. Small crystals in the presence of ATP have been obtained.

REPORTABLE OUTCOMES

Abstract accepted for presentation at Molecular Parasitism Meeting, Woodshole, September 2000.

Biswajit Pal, Brandon Pybus, Don Muccio, and Debasish Chattopadhyay.

Structure Function Analysis of *Plasmodium falciparum* 3-Phosphoglycerate Kinase.

CONCLUSIONS

In conclusion, several inhibitors have been obtained in our high throughput screens. We are waiting for the compounds from Arqule, Inc. to arrive [please see appendix VI]. Once these compounds are received, assays will be done in quadruplicate. All hits will be tested against mammalian LDH control. Compounds that show specific inhibition of *Pf*LDH will be tested against *P. falciparum* in an erythrocyte culture. This work will be done in collaboration with scientists at WRAIR.

Selected compounds will be cocrystallized with *Pf*LDH to elucidate the interaction of the small molecule with the enzyme. These structures will guide search and synthesis of newer compounds to improve potency and selectivity.

We need to invest some more efforts to molecular modeling experiments. We should investigate if there is more room for introducing more selectivity in docking. A number of compounds selected from Docking studies are being purchased for testing *in vitro* enzyme inhibitory activity. Selected compounds will be tested in parasite culture. Crystal structure of these compounds with *Pf*LDH will also be conducted side by side.

Attempts are underway to improve size of 3-pgk crystals for X-ray analysis. We will also crystallize the protein in complex with the substrate and substrate analogs and cofactor. Crystal structure of the protein and complexes will be determined in order to understand the difference structural differences between the mammalian enzyme and the parasite enzyme.

We are also preparing to clone and express *P.falciparum* hexokinase. The work will begin this year.

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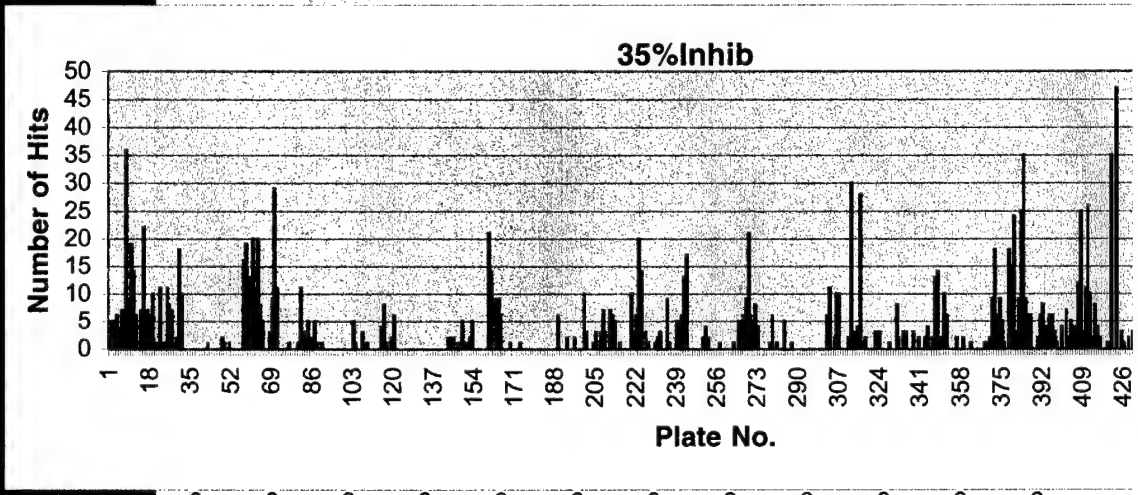
APPENDICES

I-III Summary of High Throughput Screening Results

IV-V List I and II of Compounds from Docking Study

VI Letter from ArQule

APPENDIX I


35%Inhib
Number of Hits
50
45
40
35
30
25
20
15
10
5
0
1
18
35
52
69
86
103
120
137
154
171
188
205
222
239
256
273
290
307
324
341
358
375
392
409
426
443
Plate No.

APPENDIX II

No. of Hits Total hit at > 60% inhibition level: 518 Hit Rate: 1.2%

	[redacted]												
0	[redacted]	0	0	0	0	0	0	0	0	0	0	0	0
2	[redacted]	0	0	0	0	0	0	0	0	0	0	0	0
0	[redacted]	0	0	0	0	0	0	0	0	0	0	0	0
1	[redacted]	0	0	0	0	0	0	0	0	0	0	0	0
0	[redacted]	0	0	0	0	0	0	0	0	0	0	0	0
1	[redacted]	0	0	0	0	0	0	0	0	0	0	0	0
1	[redacted]	0	0	0	0	0	0	0	0	0	0	0	0
20	[redacted]	0	0	0	1	0	0	0	0	0	0	0	0
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11	[redacted]	0	0	0	0	0	0	0	1	0	0	0	0
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0	[redacted]	0	0	0	0	0	0	0	0	0	0	0	0
3	[redacted]	0	0	0	0	0	0	0	0	0	0	0	0
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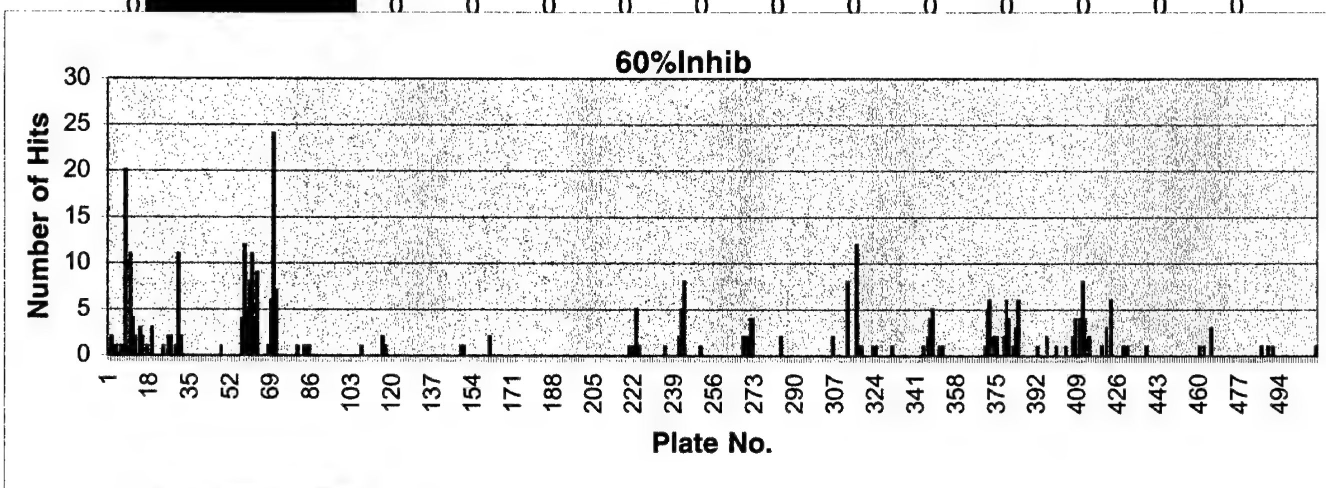

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APPENDIX III

70%Inhib
Number of Hits
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2
4
6
8
10
12
14
16
Plate No.
1
22
43
64
85
106
127
148
169
190
211
232
253
274
295
316
337
358
379
400
421
442
463
484
505

APPENDIX IV

APPENDIX 4

List of DOCK-selected inhibitor compounds and their force field scores.

1	ZEATIN_RIBOSIDE_5'-MONOPHOSPHATE,_SODIUM_SALT,_TRANS	-48.45
2	N6-([6-AMINOHEXYL]-CARBAMOYLMETHYL)-ADENOSINE_5'-DIPHOSPHATE_LITHIUM_SALT	-45.95
3	(RS)-DIHYDROZEATIN_RIBOSIDE_5'-MONOPHOSPHATE,_SODIUM_SALT	-45.72
4	N6-(6-AMINOHEXYL)ADENOSINE_5'-MONOPHOSPHATE_SODIUM_SALT	-45.44
5	8-BROMOGUANOSINE_5'-MONOPHOSPHATE_SODIUM_SALT	-44.33
6	N6-(2-ISOPENTENYL)ADENOSINE_5'-MONOPHOSPHATE,_SODIUM_SALT	-44.08
7	1,N6-ETHENOADENOSINE_5'-MONOPHOSPHATE_SODIUM_SALT	-43.55
8	H-VAL-GLY-ARG-PRO-GLU-OH	-42.71
9	KINETIN_RIBOSIDE_5'-MONOPHOSPHATE,_SODIUM_SALT	-42.29
10	NICOTINAMIDE_HYPOXANTHINE_DINUCLEOTIDE,_REDUCED_FORM_SODIUM_SALT	-41.53
11	N-ACETYL-D-GLUCOSAMINYL-BETA-(1->4)-N-ACETYLMURAMYL-L-ALANYL-D-ISOGLUTAMINE	-41.13
12	NAPHTHOCHROME_GREEN	-41.00
14	XANTHOSINE_5'-DIPHOSPHATE_SODIUM_SALT	-40.84
15	3-ACETYLPYRIDINE_HYPOXANTHINE_DINUCLEOTIDE	-40.48
16	3-PYRIDINEALDEHYDE_HYPOXANTHINE_DINUCLEOTIDE_SODIUM_SALT	-40.37
17	N6-METHYLADENOSINE_5'-MONOPHOSPHATE_SODIUM_SALT	-40.33
18	PYRENETETRASULFONIC_ACID	-40.29
19	2'-DEOXYADENOSINE-5'-TRIPHOSPHATE,_TRIAMMONIUM_SALT	-40.28
20	INOSINE_5'-MONOPHOSPHATE	-40.16
21	5'-INOSINIC_ACID_BARIUM_SALT	-40.03
22	INOSINE_5'-MONOPHOSPHATE_DISODIUM_SALT	-40.03
23	ADENOSINE_5'-PHOSPHOSULFATE_SODIUM_SALT	-39.66
24	8-(6-AMINOHEXYL)AMINO-ADENOSINE_5'-MONOPHOS-PHATE_LITHIUM_SALT	-39.66
25	ADENOSINE_5'-(BETA-THIO)_DIPHOSPHATE,_[35S]-	-39.47
26	PGLU-ASN-+CYS(+CYS)-PRO-LEU	-39.31
27	NICOTINAMIDE_HYPOXANTHINE_DINUCLEOTIDE_SODIUM_SALT	-39.16
28	3-PYRIDINEALDEHYDE_HYPOXANTHINE_DINUCLEOTIDE_SODIUM_SALT	-38.92
29	H-PRO-GLU-PRO-GLU-THR-OH	-38.85
30	ADENOSINE-5'-MONOPHOSPHORIC_ACID_DISODIUM_SALT_HEXAHYDRATE	-38.62
31	TUBERCIDIN_5'-MONOPHOSPHATE	-38.39
32	2,5-BIS(N-(4-ETHOXYPHENYL)CARBAMOYL)TEREPHTHALIC_ACID_HYDRATE	-38.53
33	PGLU-ASN-+CYS(+CYS)-PRO-LEU	-38.31
34	1,N6-ETHENO-2'-DEOXY-ADENOSINE_5'-MONOPHOSPHATE_SODIUM_SALT	-38.04
35	ADENOSINE_5'-DIPHOSPHATE_TRIS_SALT	-37.95
36	[2H6]-N(6)-(2-ISOPHENTENYL)ADENINE-9-B-D-GLUCOSIDE	-37.86
37	INOSINE_5'-DIPHOSPHORIBOSE_SODIUM_SALT	-37.77
38	N-(3-[2-FURYL]ACRYLOYL)-LEU-GLY-PRO-ALA	-37.63
39	SUBSTANCE_P_(1-5)	-37.06
40	SZS_TECH_L8/58	-36.89
41	N-ALPHA-FMOC-N-EPSILON-BIOTINYL-D-LYSINE	-36.89
42	MORDANT_BLUE_1	-36.81
43	ACETYL-(D-VAL13)-ALPHA-MSH_(11-13)	-36.77
44	LEU-ASP-VAL-PRO-SER_(HUMAN,_BOVINE,_RAT,_CHICKEN)	-36.71
45	Z-GLY-PRO-GLY-GLY-PRO-ALA-OH	-36.68
46	ALPHA-AMANITIN	-36.68
47	N-ACETYL-D-GLUCOSAMINYL-BETA-(1->4)-N-ACETYLMURAMYL-L-ALANYL-D-ISOGLUTAMINE	-36.67
48	2-BENZOYL-1-PHENYL-2A,6B-DIAZA-CYCLOPENTA(CD)PENTALENE-5,6-DICARBOXYLIC_ACID	-36.28
49	BETA,GAMMA-METHYLENEURIDINE_5'-TRIPHOSPHATE_SODIUM_SALT	-36.23
50	MURINE_CMV_PP89_(70-74)	-36.18

APPENDIX V

APPENDIX 5

List of DOCK-selected inhibitor compounds and their force field scores.

1	13,14-DIHYDRO-15-KETO_PROSTAGLANDIN_E2	-28.49
2	15(R)-15-METHYL_PROSTAGLANDIN_E2	-28.12
3	17-TRANS_PROSTAGLANDIN_E3	-27.29
4	16-PHENYL_TETRANOR_PROSTAGLANDIN_E2	-27.22
5	BOC-LYS(BIOTINYL)-OH	-27.00
6	2'-DEOXYGUANOSINE-5'-DIPHOSPHORIC_ACID,_DISODIUM	-26.83
7	15(R)-PROSTAGLANDIN_E2	-26.56
8	BOC-L-PRO-O-CH2-PHI-CH2-COOH	-26.23
9	19(R)-HYDROXY-PROSTAGLANDIN_E2	-26.04
10	17-PHENYL-TRINOR-PROSTAGLANDIN_E2	-26.03
11	5-TRANS_PROSTAGLANDIN_E2	-26.02
12	20-HYDROXY_PROSTAGLANDIN_E2	-26.01
13	PROSTAGLANDIN_E2	-25.99
14	2',3'-DIDEOXYINOSINE-5'-TRIPHOSPHORIC_ACID,_LITHIUM	-25.71
15	N-(2-PYRIDYLDITHIOPROPIONYL)-BIOCYTIN	-25.44
16	INOSINE_5'-TRIPHOSPHATE,_TRISODIUM_SALT_HYDRATE	-24.63
17	INOSINE_5'-TRIPHOSPHATE,_PERIODATE_OXIDIZED_SODIUM_SALT	-24.52
18	3-ACETYLPYRIDINE_HYPOXANTHINE_DINUCLEOTIDE	-24.40
19	2-(4-(2,2-DICARBOXY-ETHYL)-2,5-DIMETHOXY-BENZYL)-MALONIC_ACID	-24.35
20	2',3'-DIDEOXYINOSINE_5'-TRIPHOSPHATE_LITHIUM_SALT	-24.30
21	BIOTINYL-GLY-GLY-OH	-24.23
22	2',3'-DIDEOXYINOSINE_5'-TRIPHOSPHATE_LITHIUM_SALT	-24.22
23	NICOTINAMIDE_HYPOXANTHINE_DINUCLEOTIDE_PHOSPHATE,_REDUCEDFORMTETRASODIUMSALT	-24.11
24	2'-DEOXYGUANOSINE-5'-DIPHOSPHORIC_ACID,_DISODIUM	-24.01
25	BIOTIN-PROPRANOLOL_ANALOG	-23.89
26	MAYBRIDGE_NRB_00771	-23.73
27	2',3'-DIDEOXYINOSINE-5'-TRIPHOSPHORIC_ACID,_LITHIUM	-23.73
28	INOSINE-5'-TRIPHOSPHATE_DISODIUM_SALT	-23.68
29	2-(4-(2,2-DICARBOXY-ETHYL)-2,5-DIMETHOXY-BENZYL)-MALONIC_ACID	-23.59
30	INOSINE-5'-TRIPHOSPHATE,_SODIUM_SALT	-23.29
31	MAYBRIDGE_S_10318	-22.90
32	AMTHAMINE_DIHYDROBROMIDE	-22.74
33	AMTHAMINE	-22.71
34	3-(CARBOXYMETHYLAMINOMETHYL)-4-HYDROXYBENZOIC_ACID	-22.60
35	3-ISOCYANATOPROPYLTRIETHOXYSILANE	-22.43
36	MAYBRIDGE_NRB_00771	-22.41
37	DL-METHIONINE_SULFONE	-22.32
38	MONENSINE	-22.21
39	3-(CARBOXYMETHYLAMINOMETHYL)-4-HYDROXYBENZOIC_ACID	-22.60
40	7-METHOXYCOUMARIN-4-ACETYL-PRO-LEU	-21.91
41	3,5-BIS-(4-NO2-PH)-1,1-DIOXO-THIOMORPHOLINE-2,6DICARBOXYLICACIDDIMETHYLESTER	-21.89
42	MAYBRIDGE_NRB_03015	-21.79
43	16-PHENYL_TETRANOR_PROSTAGLANDIN_E2	-21.71
44	MAYBRIDGE_BTB_05614	-21.65
45	ISODESMOSINE	-21.51
46	2,4-BIS[3-(4-METHOXYANILINO)PROPYLAMINO]-3-OXO-1-CYCLOBUTENYLIUM-1-OLATE	-21.49
47	4-(4-CHLOROPHENYL)PIPERAZINYLMETHYL_PHTHALAZIN-1-ONE	-21.46
48	4-(4-PHENYLPIPERAZINYLMETHYL)PHTHALAZIN-1-ONE	-21.45
49	FLUORESCEIN-5-CARBONYL_AZIDE,_DIACETATE	-21.35
50	2,4-BIS[3-(4-METHOXYANILINO)PROPYLAMINO]-3-OXO-1-CYCLOBUTENYLIUM-1-OLATE	-21.33

1	ZEATIN_RIBOSIDE_5'-MONOPHOSPHATE_SODIUM_SALT,_TRANS	-48.45
2	N6-([6-AMINOHEXYL]-CARBAMOYLMETHYL)-ADENOSINE_5'-MONO-PHOSPHATE_LITHIUM_SALT	-47.96
3	R-(-)-DIHYDROZEATIN_RIBOSIDE_5'-MONOPHOSPHATE	-47.96
4	N6-(6-AMINOHEXYL)ADENOSINE_5'-MONOPHOSPHATE_SODIUM_SALT	-45.44
5	8-BROMOGUANOSINE_5'-MONOPHOSPHATE_SODIUM_SALT	-44.33
6	KINETIN_RIBOSIDE_5'-MONOPHOSPHATE,_SODIUM_SALT	-42.29
7	PYRENETETRASULFONIC_ACID	-40.29
8	8-(6-AMINOHEXYL)AMINO-ADENOSINE_5'-MONOPHOS-PHATE_LITHIUM_SALT	-39.66
9	ADENOSINE_5'-PHOSPHOSULFATE_SODIUM_SALT	-39.66
10	3-PYRIDINEALDEHYDE_ADENINE_DINUCLEOTIDE_SODIUM_SALT	-38.92
11	2,5-BIS(N-(4-ETHOXYPHENYL)CARBAMOYL)TEREPHTHALIC_ACID_HYDRATE	-38.53
12	TUBERCIDIN_5'-MONOPHOSPHATE	-38.39

APPENDIX VI

ARQULE

June 21, 2000

Via Facsimile

Larry J. DeLucas
Director
Center for Macromolecular Crystallography
The University of Alabama at Birmingham
1918 University Boulevard
Birmingham, AL 35294-0005

Dear Larry:

It was a pleasure speaking with you last week. As we discussed, ArQule would be willing to license active compounds identified by UAB for the lactate dehydrogenase target. In this regard, I have asked Larry Hardy to coordinate operational aspects regarding additional compound supply, etc.

I will be out of the office through July 10. I will send you a term sheet in early July.

Sincerely,

John M. Sorvillo, PhD
Vice President, Business Development

cc: Philippe Bey
Larry Hardy
Michael Rivard

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